The Etiology of False Broomrape of Tobacco

By

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TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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The false broomrape (FBR) pathogen was easily transmitted from tobacco to tobacco using any type of fasciated tissue other than the small plants formed above ground from the fasciated tissue near the soil surface. The best method for inoculation was to 1) grind FBR tissue in a sterile mortar, with additions of small amounts of 0.1 M potassium phosphate buffer at pH 7.0 until the homogenate has a consistency of a thick paste; 2) carelessly remove 20 cm tall plants from soil; 3) dust clean roots with 600 mesh carborundum and rub the roots gently with a cotton swab; 4) immerse the roots of the plants immediately in the fresh FBR homogenate and rub the roots gently with a cotton swab; and 5) transplant the inoculated plants immediately. This method gave 100% transmission. No differences were found between FBR from the United States and Central America. FBR development

is associated with the temperature most favorable for plant growth. At least 4 weeks were required for gall development. All species of *Nicotiana* tested were susceptible to FBR as were all 14 cultivars of *N. tabacum* tested. Cauliflower with FBR was observed growing in Nicaragua. Sweet potato, cabbage, and sweet pea were susceptible to FBR in greenhouse inoculations. A bacterium was isolated from FBR tissue and upon subsequent inoculations caused FBR symptoms on tobacco. This isolate, FBR-72-65, was identified as a species of *Corynebacterium* and is probably *C. fascians*. A known isolate of *C. fascians* (ICPP CF-19) caused similar symptoms on tobacco. Two colony types were observed (smooth and rough) for FBR-72-65 and ICPP CF-19 and only the smooth types were pathogenic. Results of all physiological tests with the FBR bacterium agreed with the *Bergey's Manual* description of *C. fascians* except for nitrate reduction. The known *C. fascians* isolates also were anomolous with the nitrate reduction test. The best medium for isolation of the causal bacterium was found to be one containing 40 g Difco PDA, 2 g Na_2HPO_4, 2 g NaCl, 1 g sodium citrate, 1 g asparagine, and 300 mg potassium dichromate per 1000 ml of water.

INTRODUCTION

False broomrape (FBR) is a disease of the root system of tobacco (Nicotiana tabacum L.). Symptoms of FBR include a fasciation of the root tissue in which masses of white, succulent, and irregular outgrowths form. Under certain conditions differentiation of chlorophyll containing leaves (15) and/or a complete plant may occur from the outgrowths.

Thompson (19) placed FBR tumors into the following three groups: 1) heterotopic teratomas, 2) leafy teratomas, and 3) secondary root teratomas. All the galls, except those on the main root (heterotopic teratomas), had tumor gaps and traces connecting the tumors to the vascular system of the tobacco plant.

Valleau (22) named the disease false broomrape because he first identified the disease as broomrape which is caused by Orobanchi spp., a parasitic seed plant. Thompson (19) stated that the tumors are somewhat similar to crown gall caused by Agrobacterium tumefaciens (Smith and Town) Conn and tumors that are hereditary in nature. FBR appears to have different etiology from either of these two tumor problems, however.

FBR was first reported in Kentucky on burley tobacco in 1951 (22) and has since been reported in Florida (24),

North Carolina (8), Georgia (6), and Maryland (18). It has caused little economic loss to the total tobacco crop in the United States (12), however. Jenkins (6) reported that the disease was sporadic and never caused any serious losses in Georgia and Massey (13) found the same to be true in Kentucky.

Economic losses do occur in individual fields, however. Plants in some fields in Kentucky were observed in 1969 by the author to be severely diseased (Fig. 1). It was estimated that the yield in these fields would be reduced at least 20%. The diseased plants were 1 to 3 ft in height while the healthy plants were 5 to 6 ft in height. When the root systems on the stunted plants were examined, all of them had galls ranging in diameter from 2 to 8 cm on the tap root.

The most severe losses apparently occur in Central America where FBR has been observed in Honduras, Nicaragua, El Salvador, and Panama (12, 15). In the Central American countries FBR is more severe on burley than flue-cured tobacco. The author also observed FBR on shade tobacco in the burley area of Nicaragua in 1970 and 1971. A severe outbreak in the Jalapa Valley resulted in a 28% reduction in yield (15).

Several researchers have tried and failed to isolate a causal agent of FBR (3, 8, 19). Some proposed that FBR is caused by the bacterium, _C. fascians_ Tilford (Dowson) (8, 12, 17, 19, 22). Valleau noted the similarity of this disease to leafy gall of tobacco described by Tilford (20)

Figure 1. Burley tobacco plants in a Kentucky field stunted by FBR.

and Lacey (10). Thompson (19) and Newman (17) inoculated tobacco with _C. fascians_ and obtained symptoms almost identical to FBR but were unable to complete Koch's postulates. Kelman (8) did the same but did not believe that _C. fascians_ was the causal organism because of differences in symptoms and the fact that FBR developed only after wounding, whereas _C. fascians_ did not.

C. fascians was first identified as a pathogen of sweet pea by Tilford (20) and was found to cause root fasciations, leafy galls, and several other disorders on many plants particularly on ornamental plants (1, 16). Jacobs and Mohanty (5) found that _C. fascians_ has a very wide host range ,and confirmed the existence of host specificity among strains of the pathogen. Lacey (10) isolated a bacterium associated with leafy gall of sweet peas and found that these strains were pathogenic on _Nicotiana glutinosa_ and _N. tabacum_. _Vicia faba_ L. and _Phaseolus vulgaris_ L. were susceptible to the tobacco strain (10).

FBR has the potential of becoming a serious disease on tobacco in the United States as well as on other economic crops. As a result of a review of the literature which revealed that several researchers have conflicting views as to the causal agent of the disease and subsequent personal observations of the disease in fields of tobacco in Kentucky and Central America, the author conducted research to assist in identifying the causal agent which, in turn, might aid in its control.

MATERIALS AND METHODS

Inoculation and Transmission Methods

In initial experiments inoculation techniques for transmission of FBR to tobacco were with macerated, fasciated tissue because no causal agent had been identified. Transmission of the causal agent of FBR by method described by Dukes was tried (3). The Dukes' method is 1) grind FBR tissue in a sterile mortar, with additions of small amounts of 0.1 M potassium phosphate buffer at pH 7.0; 2) grind until the homogenate has the consistency of a thick paste; 3) dust clean roots of healthy tobacco plants in the 4-5 leaf stage of growth that were carefully removed from the soil with 600 mesh carborundum and rub the roots gently with cotton swabs; 4) immerse the roots of the plants immediately in the fresh FBR homogenate and rub the roots gently with a cotton swab; and 5) transplant the inoculated plants immediately. Kelman's method (8) of inoculation was also tried. This method was simpler in that the plants' roots were wounded and immersed in a suspension of fragments of FBR tissue.

Several variations of Dukes' method were also tested. These were 1) identical procedure but the plants were pulled carelessly from the soil; 2) the roots were deliberately rubbed for injury; 3) the same

as number 1 without carborundum; 4, 5, 6) repeat the above three methods but the homogenate had been filtered through Whatman no. 1 filter paper; 7) identical to number 1 but with homogenate diluted 1:10, 1:10^2, 1:10^3, 1:10^4, and 1:10^5; 8) inject homogenate into roots with a hyperdermic syringe and 27 gauge needle; and 9) transplant in naturally and artifically infested soil (plants were pulled from soil, soil carefully washed from roots, and then rubbed with carborundum). Method 1 was used to inoculate plants in subsequent tests unless otherwise indicated, and the cultivar Hicks was also used unless otherwise indicated.

Sources and Types of FBR Tissue

Fasciated tissue was obtained from burley tobacco in Kentucky and Nicaragua (Fig. 2 and 3), cigar wrapper tobacco in Nicaragua (Fig. 4 and 5), and flue-cured tobacco in Honduras (Fig. 6). FBR was observed under natural conditions at these locations for differences in symptoms. Tissue was obtained from many fields in Nicaragua and Honduras and kept separate to screen for possible differences that might exist. Tobacco plants were inoculated with each of the above sources of diseased materials, grown in a greenhouse for several weeks, and observed for symptom expression. Tissue from the fasciated roots of a cauliflower (*Brassica olearacea* var. *botrytis* L.) plant found in Nicaragua

Figure 2. Fasciated tissue from burley tobacco plants in Kentucky infected with FBR.

Figure 3. Fasciated tissue from burley tobacco plants in Nicaragua infected with FBR.

Figure 4. Fasciated tissue from cigar wrapper tobacco in Nicaragua infected with FBR.

Figure 5. Above ground plants originating from below ground fasciated tissue caused by FBR.

Figure 6. Fasciated tissue from flue-cured tobacco in Honduras infected with FBR.

was also used to test for transmission to tobacco (Fig. 7).

Tobacco plants were inoculated with various types of fasciated tobacco tissue. This tissue was either dried or living. The living tissue was further subdivided into hardened galls (Fig. 8), succulent galls (Fig. 9), succulent leafy tissue (Fig. 10), and small plants that had developed from galls when they occurred near the soil surface (Fig. 10). The galls were also sectioned to obtain inner and outer tissue.

Effect of Temperature and Size of Plant on Fasciation

Fifteen tobacco plants of the Hicks cultivar were inoculated and placed in each of three growth chambers set at 25, 30, and 35 C. Five plants were removed at each of three time intervals of 3, 4, and 6 weeks. The plants were weighed and examined for fasciation. Any galls were then removed and weighed. Inoculated plants were compared with uninoculated checks. This experiment was repeated.

Tobacco plants of the same cultivar at heights of 10, 20, 40, 60, 80, and 100 cm were inoculated to determine the optimum size for FBR development. Inoculated plants were held in a greenhouse, and the root systems were observed at intervals of 3, 4, 5, and 6 weeks.

Figure 7. Cauliflower plant found naturally infected with FBR in Nicaragua.

Figure 8. Hardened FBR galls on roots of tobacco.

Figure 9. Succulent FBR galls on roots of tobacco.

Figure 10. Succulent leafy FBR tissue and small plants originating from underground tissue on roots of tobacco.

Host Range and Resistance Within Nicotiana Species and Cultivars of N. tabacum

Two species, *N. glutinosa* L. and *N. rustica* L., were inoculated along with the following cultivars of *N. tabacum*: Coker 80-F, F-17, N. C. 95, Hicks, Va 115, N. C. 2326, Coker 254, Coker 319, Coker 298, Speight G-7, McNair 14, Maryland 64, Burley 21, and Pennbel 69. Peanut (*Archis hypogea* L.), bean (*Phaseolus vulgaris* L.), cauliflower, sweet potato (*Ipomoea batatus* L.), cabbage (*Brassica olearacea* var. *capitata* L.), sweet pea (*Lathyrus odoratus* L.), and garden pea (*Pisum sativium* L.) were also inoculated. Plants of the above species were root inoculated and the sweet and garden pea plants were also inoculated as described by Jacobs and Mohanty (5). In this method the seeds were soaked in a slurry of FBR tissue and planted in sterile soil in the greenhouse.

Effect of Fertilization Levels on FBR Development

Twenty-five tobacco plants approximately 20 cm in height were inoculated with a slurry of FBR tissue and planted in 4-inch clay pots containing sterile soil in the greenhouse. Five different fertilization treatments were established within each group of 25 plants (5 plants/treatment). The treatments were no fertilizer, 1.5, 3, 4.5, and 6 g/pot of 3-6-9 analysis. Plants were maintained for 5 weeks following inoculation and the plants' roots, shoots, and resulting galls were weighed. Twenty-five

uninoculated plants were also planted in the soil with the five fertilizer treatments.

Determination and Isolation of Causal Agent

Since several researchers have speculated that FBR is caused by a bacterium (3, 4, 8, 12, 17, and 19), experiments were designed to eliminate other causal agents and to isolate a causal agent. A slurry of FBR tissue was made and filtered through a Whatman no. 1 filter paper several times to remove plant material. The resulting filtrate was then filtered through a 0.45μ Millipore filter to remove any bacteria that were present. Presumably a virus would pass through into the filtrate. Roots of tobacco plants were inoculated with 1) the slurry before the first filtration, 2) the filtrate obtained through the Whatman filter, and 3) the filtrate obtained through the Millipore filter. A suspension containing tobacco mosaic virus (TMV) was also passed through a 0.45μ Millipore filter and then inoculated on tobacco to determine, if indeed, a virus particle did pass through the filter. As a check the FBR filtrate obtained after Millipore filtration was plated on nutrient agar to determine if any bacteria were allowed through.

Streptomycin was added to a FBR filtrate through Whatman no. 1 paper to obtain the following concentrations: 0 ppm, 50 ppm, 100 ppm, 200 ppm, 300 ppm, and 400 ppm. Each of these was inoculated on roots of tobacco plants.

The plants were allowed to grow for 5 weeks and were observed for gall development.

Attempts were made to isolate the causal organism, and most of these attempts were directed at the isolation of a bacterium. The procedure in the first attempt was similar to that used by Tilford (20) in 1936 in his original isolation of *C. fascians* from sweet peas. A large piece of tissue was taken from the base of the fasciated growth, dipped in alcohol, flamed, and then crushed in 5 ml of sterile water. After 2 to 4 hr one drop of the liquid was transferred with a sterile pipette to a sterile dish; two drops were placed in a second dish; and three drops were placed in a third. Potato dextrose agar (PDA) at 48 C was added to the plates and held at 25 C.

This same procedure was used in other isolations except the drops were placed on solidified PDA and smeared with a sterile glass rod. This was also done without the 2 to 4 hr waiting period and repeated on nutrient agar (NA).

In another attempt the medium described by Burkholder (2) for the isolation of *C. sepidonicum*, the causal agent of tuber ring rot and wilt of potato, was used. To make Burkholder's medium approximately 300 g of sliced potatoes were boiled and the broth made up to a liter. To this was added 5 g peptone, 2 g Na_2HPO_4, 2 g NaCl, 1 g sodium citrate, 1 g asparagine, 6 g dextrose, 12 g agar.

Burkholder's medium was also tried using potassium dichromate to retard the growth of saprophytes. This was used at 100 ppm, 200 ppm, 300 ppm, and 400 ppm.

Kado and Heskett's medium D2 (9), which was proposed for isolation of Corynebacterium spp. was tried. This medium contained 10 g glucose, 4 g casein hydrolysate, 2 g yeast extract, 1 g NH_4Cl, 0.3 g $MgSO_4 \cdot 7H_2O$, 5 g LiCl, 1.2 g Tris, 40 mg polymyxin sulfate, 2 mg sodium azide, and 15 g agar per liter of water. This was adjusted to pH 7.8 with HCl before autoclaving. Polymyxin sulfate and sodium azide were added after the medium had cooled to 50 C.

Each of the types of gall tissue listed previously was used in isolation attempts with each media. The most widely used method of isolation was to macerate a young, succulent gall in sterile water, filter through Whatman no. 1 filter paper, and then dilute to 1:10, $1{:}10^2$, and $1{:}10^3$. These dilutions were added to the plates with sterile pipettes in quantities of 0.1. 0.5, and 1 ml and smeared with a sterile glass rod. The plates were incubated at 25 or 30 C.

At the beginning of the isolation experiments, different bacterial colonies were removed from the plate, purified, and inoculated to tobacco. These were also stored in sterile water and held at 10 C. Eventually, colonies visible within 24 hr were no longer isolated. Only the slower growing colonies were used, especially the ones that became visible in 3 or more days.

RESULTS

Inoculation and Transmission Methods

Dukes' method of inoculation gave 100% transmission of FBR if the slurry was used within 1 hr after preparation. When the plants used in inoculation studies were carefully removed from the soil and not injured before inoculation, 80% of the inoculated plants had FBR, whereas 100% were diseased when the plants were carelessly pulled from the soil or if they were injured by rubbing. This means that excessive injury to the plant was not required for transmission of FBR. Injury to the tap root produced a larger gall and more stunting than when the tap root was not injured.

After filtration through Whatman no. 1 filter paper to remove coarse plant parts, the filtrate was diluted $1:10^3$ before transmission dropped below 50%. The 1:1, 1:10, and $1:10^2$ dilutions gave 100% transmission (Table 1).

Inoculation of a specific root with FBR filtrate using a hypodermic needle was successful, and a dilution of $1:10^2$ gave 100% transmission. This is a very good method to use when a small amount of inoculum is available. It can also be used without disturbing the plant because soil can be carefully removed from only a small portion

Table 1. Effect of dilution of crude extract of false broomrape tissue on percent infection of tobacco roots.

Dilution	% infection
1:0	100
1:1	100
1:10	100
$1:10^2$	100
$1:10^3$	48
$1:10^4$	16
$1:10^5$	0

of the root system before inoculation.

FBR was easily transmitted using either naturally or artifically infested soil. When plants were carefully removed from the soil and transplanted in naturally or artifically infested soil, the transmission was 65%. When the plants were carelessly pulled from the soil and then planted in infested soil, the transmission percentage rose to 100%. This method produced galls on the fine lateral roots more often than did dipping the root system in the FBR homogenate.

Based on the above experiments the most efficient inoculation method was pulling the plants carelessly from the soil, washing the soil away, and then dipping the root system in a 1:10 dilution (filtered or unfiltered) of FBR homogenate. This method consistently gave 100% transmission.

Sources and Types of FBR Tissue

Field observations of FBR on burley and flue-cured tobacco disclosed no differences in symptoms. No differences were noted in symptoms in the United States or in Central America. The disease was more severe in Central America (Fig. 11) and has occurred every year for the past 10 years, whereas in the United States the disease may not appear every year. Also FBR affected plants tended to form small plants above the ground (Fig. 12) in Central America more often than in the United States.

Figure 11. Severely FBR infected root systems resulting in stunting of burley tobacco compared to uninfected plant in Nicaragua.

Figure 12. Small plants forming from fasciated tissue near the soil surface on burley tobacco in Nicaragua.

The soils of the two areas were very similar. The main difference seemed to be the lack of cold in Central America, although other reasons may exist for more severe disease in Central America.

Several fields were observed in Honduras and Nicaragua, both with and without FBR. Analysis of soil from these fields was made before the crop was transplanted. There was nothing significant about fields with or without FBR as far as elements, organic matter, or pH.

Diseased roots were brought to the University of Florida from Kentucky and Central America. Tobacco plants inoculated with FBR homogenate from various fields and maintained in the greenhouse were no different in symptomotology. Each source of fasciated tissue produced small tobacco plants from the fasciated tissue that occurs near the soil surface.

The cauliflower plants found in Nicaragua with FBR symptoms evidently were affected by the FBR causal agent. It was readily transmitted to tobacco and back to cauliflower. The symptoms on tobacco inoculated with the cauliflower homogenate were very similar to the symptoms produced with FBR tissue from tobacco.

FBR was successfully transmitted from all types of fasciated tissues but not from the shoots that developed from such tissues. Old galls were dried at room temperature and stored at room temperature for 18

months, and 50% transmissibility was still attained. FBR could be transmitted, with equal frequency, from hardened galls, succulent galls, and leafy underground tissue.

Effect of Temperature, Time From Inoculation, and Size of Plants on Fasciation

The growth of fasciated tissue was correlated with optimum conditions for growth of the tobacco plant. The results of the growth chamber experiments are given in Table 2 and Fig. 13. The optimum temperature for gall development was 25-30 C. The growth of the plants after inoculation was correlated with temperature more than with FBR, although the plants with the larger galls were slightly smaller at a given temperature than were uninoculated plants at the same temperature.

No galls were observed to develop at any of the three temperatures during the first 3 weeks following inoculation. At the end of 4 weeks galls were found at 30 C and 35 C but not at 25 C. Plants at all three temperatures had FBR at the end of 6 weeks with plants at 35 C having a smaller number and weight.

Tobacco plants 20 cm in height proved optimal for inoculation purposes. Galls developed faster on plants of this height than on plants of any of the other heights. The larger plants, 40-60 cm tall, were better for point inoculation, but for screening purposes the 20 cm plants were superior. The plants required at least 4 weeks for symptom expression regardless of size.

Table 2. Reaction of the tobacco cultivar Hicks to inoculation with flase broomrape at three temperature regimes and three time periods.

Temperature C		3 Weeks		4 Weeks		6 Weeks	
		Reaction*	Plant Wt.	Reaction	Plant Wt.	Reaction	Plant Wt.
			g		g		g
25	inoculated	-	13	-	14	+++	20
	uninoculated	-	21	-	22	-	25
30	inoculated	-	12	+	10	++++	20
	uninoculated	-	21	-	20	-	34
35	inoculated	-	12	+	9	++	11
	uninoculated	-	14	-	11	-	17

*Indicates presence (+) or absence (-) and size of galls.

Figure 13. Root systems of FBR inoculated tobacco plants grown at 25 C (right), 30 C (center), and 35 C (left).

Host Range and Resistance Within Nicotiana Species and Cultivars of N. tabacum

Three species of Nicotiana were inoculated along with several other cultivars of N. tabacum and other genera of plants. These are listed in Table 3 along with their reaction to FBR.

All three species of Nicotiana were equally susceptible to inoculation with FBR tissue. Of the 20 plants of each of the different cultivars of N. tabacum inoculated with FBR and grown in the greenhouse, five were removed from the soil, observed for FBR development, weighed, and measured at four different time intervals. No differences were noted in susceptibility among the 14 cultivars (Table 3). All plants developed fasciated tissue at the same rate and none were more retarded in overall growth than the others.

Three of the other genera of plants evaluated proved susceptible to FBR (Table 3). Cauliflower, which was observed naturally diseased with FBR in Nicaragua, was susceptible in greenhouse inoculations along with cabbage. After several attempts FBR was transmitted to sweet potato. Sweet pea plants appeared to be susceptible, although the fasciation did not develop to the extent reported by Tilford (20), Mohanty (16), Jacobs (5), and Lacey (11). Baker (1) reported Phaseolus vulgaris to be susceptible to C. fascians, but the cultivar of bean used in this work was not susceptible to FBR transmission from tobacco. Neither garden pea nor peanut was diseased.

Table 3. Reaction of several genera of plants including *Nicotiana* species and *N. tabacum* cultivars.

Genera		Reaction*
Cauliflower	*Brassica olearacea* var. *botrytis*	+
Peanut	*Arachis hypogea*	-
Bean	*Phaseolus vulgaris*	-
Sweet potato	*Ipomoea batatus*	+
Cabbage	*Brassica olearacea* var. *capitata*	+
Sweet pea	*Lathyrus odoratus*	+
Garden pea	*Pisum sativium*	-
Tomato	*Lycopersicon escutentum*	-
Tobacco	*N. rustica*	+
	N. glutinosa	+
	N. tabacum	
	Coker 80-F	+
	F-17	+
	N. C. 95	+
	Hicks	+
	Va 115	+
	N. C. 2326	+
	Coker 254	+
	Coker 319	+
	Coker 298	+
	Speight G-7	+
	McNair 14	+
	Maryland 64	+
	Burley 21	+
	Pennbel 69	+

* + indicates susceptible

Effect of Fertilization Levels on FBR Development

Fertilization had no effect on the initiation of fasciation (Table 4). Increasing the fertilization rate of the tobacco plants did not result in FBR being initiated sooner. Higher rates of fertilization did result in a faster development of the fasciated tissue once it was initiated.

Isolation of the Causal Agent

FBR was not transmitted to tobacco by using bacterial free filtrates. Neither was it transmitted using a Whatman filtrate treated with streptomycin at 100 ppm and above. When the filtrate and treated filtrate were plated on nutrient agar, few bacterial colonies grew and at 300 ppm no bacterial colonies grew. TMV particles passed through the Millipore filter since plants inoculated with TMV filtrate developed TMV. These two experiments indicate that a bacterium but not a virus is the causal agent. Ease of transmission would tend to rule out a mycoplasma as the causal agent.

The medium chosen for most of the isolation attempts contained the following per 1000 ml: 40 g Difco PDA, 2 g Na_2HPO_4, 2 g NaCl, 1 g sodium citrate, 1 g asparagine, and 100 mg potassium dichromate. Kado's medium, PDA, and NA were not successful because many saprophytic bacteria grew on them.

Table 4. Effect of fertilization levels on false broomrape development of burley and flue-cured tobacco.

Fertility level* g/pot	Tumor Weight (g)	
	Burley	Flue-cured
0	5	5
1.5	6	7
3.0	10	11
4.5	11	13
6.0	13	16

*A 3-6-9 analysis of fertilizer was used.

Seventy-three bacterial isolations were made from the different types of FBR tissue. Most were slow growing and creme, beige, yellow, or yellow-orange in color. These isolates were stored in sterile water at 10 C until the inoculated plants reacted postively or negatively after a 6 week period.

The 33rd isolate obtained (FBR-70-33) was from a gall on a plant in the greenhouse induced by tissue from burley tobacco in Kentucky. After 4 weeks four out of five tobacco plants inoculated with this isolate expressed symptoms of FBR (Fig. 14). Five more tobacco plants were then inoculated with FBR-70-33. No FBR symptoms were expressed after 7 weeks. This was repeated with the same results. Attempts to isolate the bacterium from the galls of FBR-70-33 inoculated plants were not successful.

Isolation attempts were continued, and isolate FBR-72-65 was obtained from a hardened gall of burley tobacco brought from Nicaragua. This isolate appeared on the potassium dichromate medium after 3 days as a pinpoint yellow-orange colony. This was the only colony of this type observed on any of the 20 plates that were smeared with 0.5 ml of the 1:10 dilution of the FBR homogenate previously described. This isolate stained Gram positive. All five plants inoculated with the isolate expressed FBR symptoms (Fig. 15).

After a suspect bacterium was isolated, a culture of *C. fascians* was requested from M. P. Starr, Curator of the

Figure 14. FBR symptoms produced by inoculation of tobacco roots with bacterial isolate, FBR-70-33.

Figure 15. FBR symptoms produced by inoculation of tobacco roots with bacterial isolate, FBR-72-65.

International Collection of Plant Pathogenic Bacteria, Davis, California, for comparison. This isolate of _C. fascians_ (ICPP CF-19) also produced FBR symptoms on tobacco plants. When the two bacteria, ICPP CF-19 and FBR-72-65, were cultured on Difco PDA, both turned deep orange after 5 days. When FBR-72-65 was observed with the aid of a dissecting microscope, two colony types were evident, one rough and one smooth. The majority of the colonies were of the rough type. When the _C. fascians_ isolate obtained from Starr was observed under the microscope, the same two colony types were observed. But in this instance the majority were of the smooth type. The smooth isolate was then designated FBR-72-65-1S and the rough isolate, FBR-72-65-1R.

Tobacco plants were inoculated with each colony type from both ICPP CF-19 and FBR-72-65. In both instances the smooth isolates proved pathogenic and the rough ones did not. Selective development of the rough isolate in culture probably explains why the original FBR-72-65 and FBR-70-33 seemed to lose pathogenicity on subsequent inoculations. The smooth colony type was reisolated from galls caused by inoculation with FBR-72-65, and subsequent inoculations produced FBR symptoms.

C. fascians grew on Burkholder's medium with additions of up to 300 ppm potassium dichromate which severely retarded growth of saprophytic bacteria and fungi. With this medium reisolation of FBR-72-65 from

a gall resulting from inoculation by FBR-72-65 was accomplished.

Identification of FBR-72-65

Physiological tests were undertaken using the known *C. fascians* (rough and smooth) and FBR-72-65-1S and -1R. These and the results are listed in Table 5 along with the characterization from *Bergey's Manual*. All test results of the FBR-72-65-1S and -1R agreed with the results of the known *C. fascians* (rough and smooth). The nitrate reduction tests did not agree with *Bergey's Manual*. Based on these tests and comparisons with the known C. fascians, FBR-72-65 is a species of *Corynebacterium* and in all likelihood is *C. fascians*.

Table 5. Results of physiological, Gram stain, and optimum temperature tests conducted on known *C. fascians* and bacterial isolate, FBR-72-65.

Test	*C. fascians* Bergey's Manual	*C. fascians* ICPP CF-19	FBR-72-65
Gelatin lignification	-	-	-
Nitrate reduction	+	-	-
Starch hydroylis	-	-	-
Hydrogen sulfide	+	+	+
Litmus milk blued	+	+	+
Acid formation			
Dextrose	+	+	+
Maltose	+	+	+
Xylose	+	+	+
Lactose	-	-	-
Gram stain	+	+	+
Optimum temperature	25-28 C	25-30 C	25-30 C

DISCUSSION

Tobacco plants may be severely stunted and losses in yield may result if the tobacco plants become infected with FBR soon after transplanting. The size plant used for transplanting is easily inoculated with the causal agent of FBR. If the seedbed is contaminated, this may be the source of inoculum for previously uninfested fields. In greenhouse experiments FBR was easily transmitted when tobacco plants were planted in soil from a FBR infested field. Old, dried, fasciated tissue is a good source of FBR inoculum.

No differences were found between any of the sources of FBR. When they were inoculated on tobacco plants in a greenhouse, the symptoms expressed were identical. All would form small tobacco plants from fasciated tissue near the soil surface. Thompson (19) did not find this to be true in earlier experiments.

Tobacco plants could be inoculated with macerated tissue from any type of fasciated tissue and FBR was transmitted. This inoculum could be filtered and diluted $1:10^4$ and still be viable. All bacteria could be removed from this filtrate, and FBR symptoms could still be reproduced on tobacco plants.

This disease has been reported in Florida (24),

North Carolina (8), Georgia (6), Maryland (18), and Central America (12, 15). Many researchers have tried and failed to determine the causal agent (3, 8, 17). It was proposed that FBR is caused by the bacterium *C. fascians* (8, 12, 17, 19, 22). Working on this premise, organisms other than a bacterium were ruled out, and isolation techniques for the isolation of a bacterium were employed using a medium similar to one suggested by Burkholder (2). The medium was the same except Difco PDA and 300 mg potassium dichromate were used. On this medium *C. fascians* is allowed to grow without being overgrown by faster growing saprophytic organisms. After several attempts at isolation a bacterium was isolated that reproduced FBR symptoms when inoculated on tobacco. This same bacterium was reisolated.

Several physiological tests were conducted on the isolate, FBR-72-65, from fasciated tobacco tissue as well as a *C. fascians* isolate from M. P. Starr. They all agreed with the results in *Bergey's Manual* except the nitrate reduction test. Starr's *C. fascians* isolate did not agree with *Bergey's Manual* in this respect. Based on these tests and growth characteristics the isolate was identified as *C. fascians*.

SUMMARY

FBR is a very easily transmitted disease and no extra or excessive injury to the root systems of tobacco plants is necessary. Ample injury is inflicted to a tobacco plant during normal transplanting procedures.

Any type of gall tissue can be used to transmit the causal agent of FBR. Dried tissue is also effective. This means that FBR can easily be carried over from one growing season to another.

The temperature optimum for plant growth, 25-30 C, is optimum for FBR development, and almost any size or age of plant can be infected. At least 4 weeks are required for expression of symptoms.

All Nicotiana species tested were susceptible to FBR as well as some 14 cultivars of N. tabacum. Cauliflower was found to be naturally infected with FBR in Central America. This FBR was easily transmitted to tobacco and back to cauliflower. Sweet potato, cabbage, and sweet pea were also suscepts to the disease.

Increasing fertilization levels did not cause FBR to be initiated sooner but did result in a faster development of the fasciated tissue. This could explain why FBR is more severe on burley tobacco than flue-cured since burley received extremely high rates of fertilizer.

A bacterium was isolated from fasciated tissue from Central America, and this bacterium produced FBR symptoms on tobacco plants in the greenhouse. The same bacterium was reisolated from these plants using a medium containing 40 g Difco PDA, 2 g Na_2HPO_4, 2 g NaCl, 1 g sodium citrate, 1 g asparagine, and 300 mg potassium dichromate per liter of water. This bacterium was identified as Corynebacterium fascians. Two colony types were present, a rough colony which was not pathogenic and a smooth type which was pathogenic.

More work is need with this disease and the causal agent. Since the causal agent was found to be a bacterium, a control method can probably be developed. In as much as the disease is not at present a wide spread economic problem, the control would probably be with chemicals and/or crop rotation. The possibility of a wild host should also be investigated as well as cultural and environmental conditions that may favor the disease and influence its severity.

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BIOGRAPHICAL SKETCH

Wayne Clark Mixson was born on a farm in Marion County, Florida, on November 29, 1944. He completed twelve years of school at Reddick, Florida, and was graduated from North Marion High School in 1962. He attended the University of Florida at Gainesville. In 1966 he received the degree of Bachelor of Science in Agriculture with a major in agronomy and in 1969 the degree of Master of Science in Agriculture with a major in agronomy. In September, 1969, he began studies toward the degree of Doctor of Philosophy. He is a member of the American Phytopathological Society, the American Society of Agronomy, Crop Science Society of America, and the Florida Turf-Grass Association. He is employed as a research agronomist by O. M. Scott and Sons, Apopka, Florida.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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